Peer Review
Experimental Toxicology Division
N H E E R L

WATER

Drinking Water Disinfection Byproduct Pharmacokinetics: Linking Brominated Trihalomethane Exposure to Health Effects

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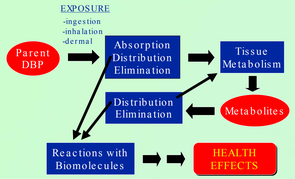
Key Issues for Brominated THMs

- BrTHMs are among the most prevalent disinfection byproducts (DBPs) in drinking water
- Bromodichloromethane is the most potent carcinogen among the THMs in rodents
  - Colon and renal carcinomas in rats
  - Kidney and liver tumors in mice
- Concordance of epidemiological and animal research findings (colon cancer and reproductive toxicity)
- Mutagenicity/genotoxicity of BrTHMs:
  -GSH-dependent metabolic pathway
- Identification and description of pharmacokinetics and key events/pathways
  - Compare rodents and humans

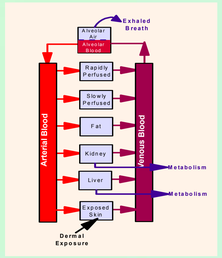
DBP PHARMACOKINETICS



RESEARCH OBJECTIVES

1. Development of physiologically based pharmacokinetic (PBPK) models for bromodichloromethane (BDCM).
2. Evaluate BDCM pharmacokinetics in humans, including in vivo exposures and in vitro metabolism by key enzymes.
3. Characterize the mutagenic glutathione transferase theta pathway, including studies of the DNA reactivity of intermediates.
4. Assess the relationship of CYP2E1 and GST T1-1 as competing pathways in target tissues for BDCM-induced cancer.

PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING FOR BROMODICHLOROMETHANE



Selected PBPK model parameters for BDCM and chloroform

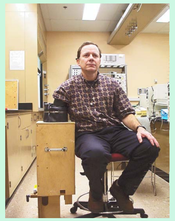
Parameter	BDCM	Chloroform[a]
Partition Coefficients		
Fat/Air	526	203
Liver/Air	30.6	21.1
Metabolic Constants		
V_{max_C} (mg/hr/kg)	12.8	6.8
K_m (mg/L)	0.5	0.5

[a]Corley et al. (1990) Toxicol. Appl. Pharmacol. 103, 512.

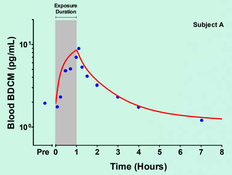
BDCM Rat Model Summary

- Higher partition coefficients demonstrate greater tissue uptake of BDCM compared to chloroform
- BDCM is metabolized to reactive intermediates at a faster rate than chloroform
- The model was able to accurately predict:
  - Blood and tissue concentrations of BDCM after oral and inhalation dosing
  - Metabolite (bromide ion) production after dosing

BROMODICHLOROMETHANE PHARMACOKINETICS IN HUMANS



PBPK modeling of human blood BDCM concentrations during and after dermal exposure



In Vivo Exposure Summary

- Volunteers were exposed either dermally or orally to water containing BDCM at a level normally found in finished drinking water.
- Significantly higher blood concentrations of BDCM were attained with dermal exposure than with oral consumption (40 -100 -fold difference).
- An initial PBPK modeling effort was able to predict the blood concentration profile.

IN VITRO METABOLISM

Summary of Metabolic Constants for Recombinant Cytochrome P450's

Isoenzyme	Metabolic Parameter[a]			
	Human		Rat	
	K_m	k_{cat}	K_m	k_{cat}
CYP2E1	3.5 (0.5)	2.3 (0.1)	4.6 (0.3)	3.5 (0.1)
CYP1A2	94 (29)	4.6 (0.7)	355 (109)	19.8 (4.4)
CYP2A6	206 (62)	1.4 (0.3)	—[b]	—
CYP3A4	238 (44)	16.6 (1.9)	—	—
CYP2B1	—	—	127 (16)	0.89 (0.06)

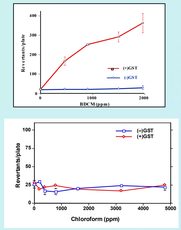
[a] Units of Km and k_{cat} are μM and mol BDCM·(mol P450 ·min)$^{-1}$ respectively.
[b] Enzyme not present.

- The kinetics of CYP2E1- mediated BDCM metabolism are similar in humans and rats.
- We discovered that CYP1A2, CYP3A4, and CYP2A6 also metabolize BDCM.
- BDCM was not metabolized by human CYPs 2B6 and 2D6 or by rat CYPs 3A1 and 2C11.

DISCOVERY AND CHARACTERIZATION OF A GENOTOXIC METABOLIC PATHWAY FOR BROMINATED THMs

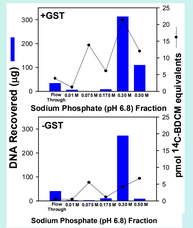
GSH S-transferase Salmonella TA1535 Mutagenicity Assay System

Revertants produced in Salmonella TA1535 (+)GST and (-)GST with BDCM and chloroform



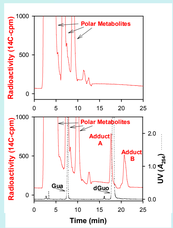
- BrTHMs, but not chloroform, are metabolized to mutagens by the GST-theta enzyme.
- CHBr Cl and CHBr are more potent mutagens in this assay than BDCM.
  - Relative potency corresponds with ability to induce preneoplastic colon lesions
- These gene mutations are very specific: GC → AT transitions
- The GST T1 -1 enzyme required for the pathway is present in humans; it is polymorphically expressed in people
  - Human enzyme expressed in urinary and GI tracts
  - Expression may determine susceptibility

GST T1-1-Dependent DNA Binding by BDCM



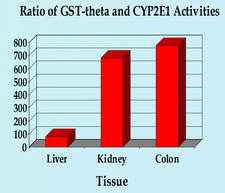
- Hydroxyapatite chromatography demonstrated that DNA was covalently modified *in vitro* by GST theta-mediated metabolism of ^{14}C-BDCM.

GST T1-1-Catalyzed Formation Of Deoxyguanosine Adducts



- Formation of adducts A and B was dependent on the presence of dGuo and GSH; adduct B was completely dependent on GST T1-1.

Relative Activities (nmol/min/g) of GST T1-1 and CYP2E1 in Different Tissues of the Rat



- This graph demonstrates that higher ratios of GST theta:CYP1E1 activities are found in the cancer target tissues (kidney and colon) for BDCM in rats than in the liver, where no cancers were induced by BDCM.

SUMMARY

- A PBPK model for BDCM has been developed that will be further parameterized and calibrated for humans using *in vivo* and *in vitro* human pharmacokinetic data.
- Human volunteer studies have demonstrated that much higher blood levels of BDCM occurred following dermal exposures than after drinking water exposures.
- New enzymes have been shown to metabolize BrTHMs. These include CYP1A2, CYP3A4, CYP2A6, and GST T1-1.
- A genotoxic metabolic pathway for BrTHMs has been discovered that is mediated by glutathione S-transferase theta 1-1. This pathway produces intermediates that covalently bind DNA (specifically producing deoxyguanosine adducts), thus leading to gene mutations.
- Similar kinetics of CYP2E1- and GST T1-1-mediated BDCM metabolism in rats and humans suggest that the rat is a relevant animal model for BDCM.
- Recent findings suggest that the metabolites derived from BrTHMs via the GST pathway are more mutagenic than those produced by methylene chloride. The rate of BrTHM-GSH conjugation is, however, less than that of methylene chloride. Additional metabolites have now been identified as products of BrTHM-GSH reactions, including *S-formyl*-GSH and formate.
- Target tissues for BDCM-induced carcinomas have higher ratios of GST T1-1:CYP2E1 activity.

IMPACT

- The PBPK model for bromodichloromethane can be used for interspecies, route-to-route, and high-to-low dose extrapolation for risk assessment. The generation of *in vivo* human data provides a unique opportunity to calibrate the model and test the predictive utility of kinetic parameters derived from *in vitro* experiments.
- Pharmacokinetic and mutagenicity findings indicate that the BrTHMs are of greater concern as potential human carcinogens than is chloroform.
- Brominated THMs are activated to mutagens by the GST T1-1 enzyme, which is polymorphically expressed in humans and may therefore be an important determinant of susceptibility to the genotoxic and potential carcinogenic effects of brominated THMs.

FUTURE DIRECTIONS

- Development of a human PBPK model for BDCM using *in vitro* data to initially set kinetic parameters and the *in vivo* data to test and calibrate the model
- Assess DNA reactivity of the other brominated THMs
- Relate covalent modification of DNA by BrTHMs in cancer target tissues to metabolic rates
- BrTHM pharmacokinetic studies relevant to reproductive endpoints, including pregnancy loss in nonhuman primates
- Pharmacokinetic studies of nitrohalomethanes, a new potent genotoxic class of DBPs generated by chloramination

SOLVING AGENCY PROBLEMS